

Application No. 10/559,097
Amendment dated July 22, 2008
In Reply to Office Action of January 24, 2008
Attorney Docket No. 4559-053584

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 4, line 7 with the following amended paragraph:

The NHX protein is a sodium antiporter. OsNHX proteins are Na⁺/H⁺ antiporters located at the vacuolar membranes and function in excluding Na⁺ from the cytosol to the vacuole in response to the electrochemical H⁺ gradient. A characteristic of mammalian Na⁺/H⁺ antiporters is their inhibition by amiloride. A putative amiloride binding site has been defined in HsNHX1 : DVFFLFLPPI (SEQ ID NO: 31). This motif is highly conserved in yeast and plant NHX genes (Gaxiola et al. 1999 PNAS; Yokio et al. Plant J. 2002 Jun; 30 (5): 529-539). NHX proteins may therefore readily be identified by the presence of the following consensus sequence for the amiloride (sodium) binding site: FFXXLLPPII (SEQ ID NO: 32), where X may be any amino acid.

Please replace the paragraph bridging pages 4 and 5 of the specification with the following amended paragraph:

The expression "NHX protein" as used herein refers to a protein having: (i) the following consensus sequence: FFXXLLPPII (SEQ ID NO: 32); and (ii) having (in increasing order of preference) at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more sequence identity to the sequence represented by SEQ ID NO: 2; and/or (iii) having Na⁺/H⁺ activity. Any nucleic acid encoding a protein falling within the aforementioned definition may be suitable for use in the methods of the invention. NHX proteins falling under the aforementioned definition are referred to herein as "essentially similar" to the sequence represented by SEQ ID NO 2. A gene encoding an NHX protein is a gene essentially similar to the sequence represented by SEQ ID NO 1. The term "essentially similar to" SEQ ID NO 1 or SEQ ID NO: 2 includes SEQ ID NO 1 or SEQ ID NO 2 itself and includes homologues, derivatives and active fragments of SEQ ID NO: 2 and includes portions of SEQ ID NO: 1 and sequences capable of hybridising to the sequence of SEQ ID NO: 1. The sequence of SEQ ID NO 1 has previously been deposited in the GenBank under the accession number AB021878 and

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the corresponding protein, SEQ ID NO 2, has been deposited in GenBank under accession number BAA83337.

Please replace the paragraph beginning on line 16 of page 5 of the specification with the following amended paragraph:

It should be clear that the applicability of the invention is not limited to use of a nucleic acid represented by SEQ ID NO 1 nor to the nucleic acid sequence encoding an amino acid sequence of SEQ ID NO 2, but that other nucleic acid sequences encoding homologues, derivatives or active fragments of SEQ ID NO 2 may be useful in the methods of the present invention. Nucleic acids suitable for use in the methods of the invention include those encoding NHX proteins according to the aforementioned definition, i. e. having: (i) the following consensus sequence: FFXXLLPPII (SEQ ID NO: 32); and (ii) having (in increasing order of preference) at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more sequence identity to the sequence represented by SEQ ID NO: 2; and/or (iii) having Na⁺/H⁺ activity.

Please replace the paragraph beginning on line 10 of page 8 with the following amended paragraph:

Methods for the search and identification of homologues of an NHX protein would be well within the realm of a person skilled in the art. The search and identification of homologous genes involves the screening of sequence information available, for example, in public databases, that include but are not limited to the DNA Database of Japan (DDBJ) (<http://www.ddbj.nig.ac.jp>); Genbank (<http://www.ncbi.nlm.nih.gov/web/Genbank/Index.html>); and the European Molecular Biology Laboratory Nucleic Acid Sequence Database (EMBL) (<http://www.ebi.ac.uk/ebi-does/embl-db.html>) or versions thereof of the MIPS database. A number of different search algorithms have been developed, including but not limited to the suite of programs referred to as BLAST programs. There are five implementations of BLAST, three designed for nucleotide sequence queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, Trends in Biotechnology: 76-80, 1994; Birren et al., GenomeAnalysis, 1:543, 1997). Such methods

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involve alignment and comparison of sequences. The BLAST algorithm calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information. Other such software or algorithms are GAP, BESTFIT, FASTA, and TFASTA. GAP uses the algorithm of Needleman and Wunsch (J. Mol. Biol. 48: 443-453, 1970) to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps.

Please replace the first full paragraph on page 9, beginning at line 6, with the following amended paragraph:

The above-mentioned analyses for sequence homology is preferably carried out on a full-length sequence, but may also be based on a comparison of certain regions such as conserved domains. The identification of such domains, would also be well within the realm of the person skilled in the art and would involve, for example, a computer readable format of the nucleic acids of the present invention, the use of alignment software programs and the use of publicly available information on protein domains, conserved motifs and boxes. This information is available in the PRODOM (<http://www.biochem.ucl.ac.uk/bsm/dbbrowser/ji/prodomsrehji.html>), PIR (<http://pir.georgetown.edu/>) or pFAM (<http://pfam.wustl.edu/>) database. Sequence analysis programs designed for motif searching may be used for identification of fragments, regions and conserved domains as mentioned above. Preferred computer programs include, but are not limited to, MEME, SIGNALSCAN, and GENESCAN. A MEME algorithm (Version 2.2) may be found in version 10.0 of the GCG package; or on the Internet site <http://www.sdsc.edu/MEME/meme>. SIGNALSCAN Version 4.0 information is available on the Internet—site <http://biosei.cbs.umn.edu/software/sigsean.html>. GENESCAN may be found on the Internet-site <http://gnomie.stanford.edu/GENESCANW.html>.

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Please replace the first full paragraph on page 10, beginning at line 7, with the following amended paragraph:

Suitable homologues are those having: (i) the following consensus sequence: FFXXLLPPII (SEQ ID NO: 32); and (ii) having (in increasing order of preference) at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more sequence identity to the sequence represented by SEQ ID NO: 2; and/or (iii) having Na⁺/H⁺ activity. Nucleic acids encoding such homologues are useful in the methods of the invention.

Please replace the first full paragraph on page 12 of the specification, beginning at line 10, with the following amended paragraph:

"Active fragments" of an NHX protein encompasses at least five contiguous amino acid residues of a protein, which residues retain similar biological and/or functional activity to the naturally occurring protein. A preferred fragment of an NHX protein is a C-terminal truncated version of the NHX protein, lacking one or more or all of the 100 last amino acids. Other preferred fragments are fragments of the NHX protein starting at the second or third or further internal methionine residues. A further preferred fragment comprises the motif of SEQ ID NO: 32, namely: FFXXLLPPII, where X can be any amino acid and/or wherein the protein has Na⁺/H⁺ activity.

Please replace the second full paragraph on page 12 of the specification, beginning at line 18, with the following amended paragraph:

Advantageously, the method according to the present invention may also be practised using portions (fragments) of DNA or of a nucleic acid sequence. The term "DNA fragment or DNA segment or portion" refers to a piece of DNA derived or prepared from an original (larger) DNA molecule, which DNA fragment or segment, when expressed in a plant, gives rise to plants having modified growth characteristics. The DNA fragment or segment may comprise many genes, with or without additional control elements, or may contain just spacer sequences etc. The fragment is preferably greater than 66 nucleotides and further preferably encodes a protein comprising the motif of SEQ ID NO: 32: FFXXLLPPII, where X can be any amino acid and/or

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wherein the protein has Na⁺/H⁺ activity. In the case where portions are to be used as molecular markers, or in other breeding applications, the portion may of course be much shorter.

Please replace the paragraph bridging pages 13 and 14 of the specification with the following amended paragraph:

The methods according to the present invention may also be practiced using an alternative splice variant of a nucleic acid sequence encoding an NHX protein, for example a splice variant of SEQ ID NO 1. The term "alternative splice variant" as used herein encompasses variants of a nucleic acid sequence in which introns and/or exons have been excised and/or replaced and/or added. Such variants will be ones in which the biological activity of the protein remains unaffected, which may be achieved by selectively retaining functional segments of the protein. Such splice variants may be found in nature or may be manmade. Methods for making such splice variants are well known in the art. Therefore according to another aspect of the present invention, there is provided, a method for improving the growth characteristics of monocotyledonous plants, comprising increasing expression in a plant of a nucleic acid sequence encoding an alternative splice variant of an NHX protein and/or by modulating activity of a protein encoded by a splice variant of an NHX protein. The splice variant preferably encodes a protein comprising the motif of SEQ ID NO: 32: FFXXLLPPII, where X can be any amino acid and/or Na⁺/H⁺ activity.

Please replace the first full paragraph on page 14 of the specification, beginning at line 7, with the following amended paragraph:

Another method for modifying plant growth characteristics resides in the use of allelic variants of a gene essentially similar to SEQ ID NO 1. Allelic variants exist in nature and encompassed within the methods of the present invention is the use of these natural alleles. "Allelic variants" are defined herein comprise single nucleotide polymorphisms (SNPs) as well as small insertion/deletion polymorphisms (INDELs ; the size of INDELs is usually less than 100 bp). SNPs and INDELs form the largest set of sequence variants in naturally occurring polymorphic strains of most organisms. Differences between alleles are naturally occurring differences between the genes of different plants of the same species. These differences may be substitution and/or addition and/or deletion of for example 1, 2, 3 or more base pairs. The allelic variant

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preferably encodes a protein comprising the motif according to SEQ ID NO: 32: FFXXLLPPH,
where X can be any amino acid and/or Na+/H+ activity.